PATENT
Attorney Docket No. 400684/SOEI

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

ISHIBASHI et al.

Art Unit: Unassigned

Application No.: Unassigned

Examiner: Unassigned

Filed: February 5, 2001

For: METHOD FOR SELECTIVELY SEPARATING LIVE CELLS EXPRESSING A SPECIFIC GENE

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to the examination of the above-identified patent application, please enter the following amendments and consider the following remarks. Copies of the pages showing the requested changes are attached.

IN THE SPECIFICATION:

Insert the following paragraph on Page 14, after BRIEF DESCRIPTION OF THE DRAWINGS:

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawings(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Replace the paragraph beginning at page 2, line 12 with:

However, for the cell labeling method by microinjection described above, the method can not label many cells at once. The number of the cells to which the labeled antibodies can be introduced for one experiment is at most ten or less. In addition, it is not easy to introduce the solution of a polymer with the molecular weight greater than 120,000 like an antibody with high concentration into the cell because of its high viscosity. Therefore, microinjection is impractical to label the sufficient number of objective cells efficiently.

Replace the paragraph beginning at page 3, line 15 with:

A typical example, where the situation described above exists and when it is difficult to separate the objective live cells selectively, includes the case where cells secreting a specific cytokine are selectively separated using the cytokine as a selection marker.

Replace the paragraph beginning at page 3, line 21 with:

When an antigen invades an organism, helper T cells (CD4+ T cells) that recognize the antigen as a foreign matter are activated, and then they will be differentiated into TH1 and TH2, which have different immune functions from each other: TH1 (T Helper 1) which is responsible for cellular immune functions, e.g., activation of macrophages to remove foreign matters by phagocytosis; and TH2 (T Helper 2) which has humoral immune functions, e.g., activation of B cells to produce antibody molecules to neutralize foreign matters and (See Fig. 94). TH1 and TH2 produce cytokines, interleukin-2 (IL-2) and interleukin-4 (IL-4), respectively. In the healthy state,

TH1 and TH2 control each other's functions and keep a balance. However, once this relationship is disrupted, it causes various infections or autoimmune disorders.

Replace the paragraph beginning at page 4, line 19 with:

For example, it has been reported that the tissue infiltration which is dependent on adhesive molecules, P- and E-selectin, is observed specifically with human TH1 (Austrup, F. et al. Nature, 385, 81-83, 1997). This suggests that ligands adhering specifically to the selectins are present on TH1 cell surfaces. However, when reactivity for P- and E-selectin is examined by flow cytometry, the results are TH1:TH2=131:52 for P-selectin, and TH1:TH2=668:88 for E-selectin; therefore, the specificity is not complete. These results can be interpreted as reflecting the fact that particularly notable P- and E-selectin ligand expression is induced in TH1 under physiological conditions unique to inflamed tissues (such as skin and joints).

Replace the paragraph beginning at page 11, line 17 with:

a second step of labeling the mRNA with the marker to obtain a live cell group containing live cells having the labeled mRNA; and

Replace the paragraph beginning at page 11, line 20 with:

a third step of detecting the labeled mRNA to identify the live cells having the labeled mRNA and separating the identified live cells selectively from the live cell group obtained in the second step.

Replace the paragraph beginning at page 11, line 24 with:

In the method for selectively separating live cells which have expressed a specific gene according to the present invention, it is preferable that the marker in the first step is a probe which has a base sequence complementary to the mRNA and has been labeled with a fluorescent dye, the labelcd mRNA in the second step is a hybrid of the probe and the mRNA, and the selective separation in the third step is performed by irradiating light to thc live cell group containing live cells having the hybrid, identifying live cells which cause a change in fluorescence of said fluorescent dye based on formation of the hybrid, and separating the identified live cells from the live cell group.

Replace the paragraph beginning at page 12, line 12 with:

It is also preferable that the probe comprises a first probe and a second probe, the first probe and the second probe have base sequences capable of hybridizing to the mRNA adjacently, the first probe is labeled with an energy donor fluorescent dye and the second probe is labeled with an energy acceptor fluorescence dye, and the change in fluorescence is caused by fluorescence resonance energy transfer (FRET) from the energy donor fluorescence dye of the first probe to the encrgy acceptor fluorescence dye of the second probe.

Replace the paragraph beginning at page 12, line 23 with:

In addition, in the method for selectively separating live cells which have expressed a specific gene according to the present invention, it is prefcrable that the selective separation in the third step of thc live cells based on the changes in fluorescence is performed by a cell sorter (FACS).

Replace the paragraph beginning at page 13, line 3 with:

It is also preferable that the mRNA is an mRNA encoding a cytokine. It is more preferable that the mRNA is an mRNA encoding interleukin-2 (IL-2) and the first probe is a probe having a base sequence set forth in SEQ ID NO: 9 in the Sequence Listing and further that the second probe is a probe having a base sequence set forth in SEQ ID NO: 10 in the Sequence Listing.

Replace the paragraph beginning at page 13, line 11 with:

It is also preferable that the mRNA is an mRNA encoding interleukin-4 (IL-4) and the first probe is a probe having a basc sequence set forth in SEQ ID NO: 17 in the Sequence Listing and further that the second probe is a probe having a base sequence set forth in SEQ ID NO: 18 in the Sequence Listing.

Replace the paragraph beginning at page 13, line 17 with:

In the present invention, it is preferable that the live cells selectively separated in the third step are T Helper 1 (TH1) or T Helper 2 (TH2) cells.

Replace the paragraph beginning at page 79, line 23 with:

The IL-2 expression-induced and -uninduced Jurkat E6-1 cells ($5x10^5$ cells/ml) werc washed with PBS(-) three times, and suspended with 1 ml of PBS(-), and the suspension was mounted on 12 mm of a cover glass (poly-L-lysine was coated on the bottom) to prepare a monolayer of cells. The cells were exposed to 0.5% Triton-X100 solution for 90 seconds at room temperature to permeabilize the cells. After the

permeabilized cells were quickly washed with PBS(-), 10 μM (final concentration) of probes corresponding to SEQ ID NO: 1-10 in Table 1 (unlabeled with dye), oligo dA (deoxythymidine oligonucleotide, unlabeled with dye) or oligo dA (deoxyadenine oligonucleotide, unlabeled with dye) were added, and incubated for one hour at room temperature. The cells were washed with PBS(-) quickly, and fixed with 4% paraformaldehyde solution at room temperature for 15 minutes.

Replace the paragraph beginning at page 94, line 22 with:

The cells fixed at the bottom of the dish were washed with PBS(-) three times and treated with 0.1% Triton X-100/PBS solution at room temperature for 5 minutes to permeabilize the cells, and the permeabilized cells were washed with PBS(-) three times and treated with 0.2N HCl at room temperature for 10 minutes. After washing the monolayer cells with PBS(-), they were incubated with 1 μg/ml of proteinase K/ PBS solution for 5 minutes at 37 °C. After the monolayer cells were washed with PBS(-), they was fixed again with 4% of paraformaldehyde / PBS (pH 7.4) for 30 minutes. The fixed cells were washed twice with 2 mg/ml of glycine/PBS for 15 minutes, and treated with 50% deionized formamide/2 x SSC solution (solution A, described hereunder) for 30 minutes; the hybridization solution (50% deionized formamide, 5 x denhardt, 2 x SSC, alkaline denatured probes for IL-2 RNA (1 μg/ml)) was prepared, denatured at 90 °C for 10 minutes, and then the monolayer cells were ice-cooled. Adding 100 μl of the hybridization solution to the cells, they were incubated at 45 °C overnight.

Replace the paragraph beginning at page 111, line 1 with:

A suspension of the fluorescent probes-introduced cells obtained in (23) was applied to a flow cytometer (FACSCalibur). The excitation light of a donor dye (Bodipy) was irradiated to the cells to detect FRET fluorescence emitted from acceptors (Cy5) based on the hybridization in the same manner as (19), and then relative fluorescence intensity of Bodipy or Cy5 was shown as FL1-Height or FL3-Height, respectively in dot-plots diagram. Among these plots, a group of cells with the highest value of FL3-Height was designated as R2 (Fig. 77). On the other hand, a group of typical human lymphocytes in the points of cell size (FSC-Height; forward-scattering light) as well as the complexity in the intrastructure (SSC-Height; side-scattering light) was designated as R1 (Fig. 76). Cells belonging to both R1 and R2 were selectively separated using a cell sorting function.

REMARKS

The application has been amended to improve the form without adding new matter.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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